
**NUCLEAR EXTRUSION AND INTRACISTERNAL
INCLUSIONS IN THE RABBIT BLASTOCYST**

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Observations on the transfer of material from nucleus to cytoplasm are abundant in the literature of light microscopy. Although in many cases the exchange of substances has been inferred from fixed and stained material, the transfer of proteins (12) and nucleic acids (7) has also been described from biochemical and radioautographic studies. In some cases, loss of nuclear material to the cytoplasm is probably an early stage in cell degeneration (4, 13, 18, 21). In other cases it is undoubtedly a normal concomitant of nuclear-cytoplasmic interaction (10, 11).

The electron microscope has demonstrated the presence of annuli or "pores" in the nuclear membrane (22, 23) which, at least in some cases (1, 2, 16, 24), appear to be sites of nuclear-cytoplasmic exchange. Also a variety of blebs and outpocketings of the nuclear envelope have been observed, and their possible role in the transfer of nuclear material to the cytoplasm has been stressed. These include the outer membrane of the nuclear envelope in a variety of species (5, 19, 22) or the outpocketing of both layers as in *Drosophila* (6, 20). The possibility that "membrane flow" may carry material from nucleus to cytoplasm has also been suggested (3). The significance of all these structures in terms of actual cell function is at present largely obscure.

MATERIAL AND METHODS

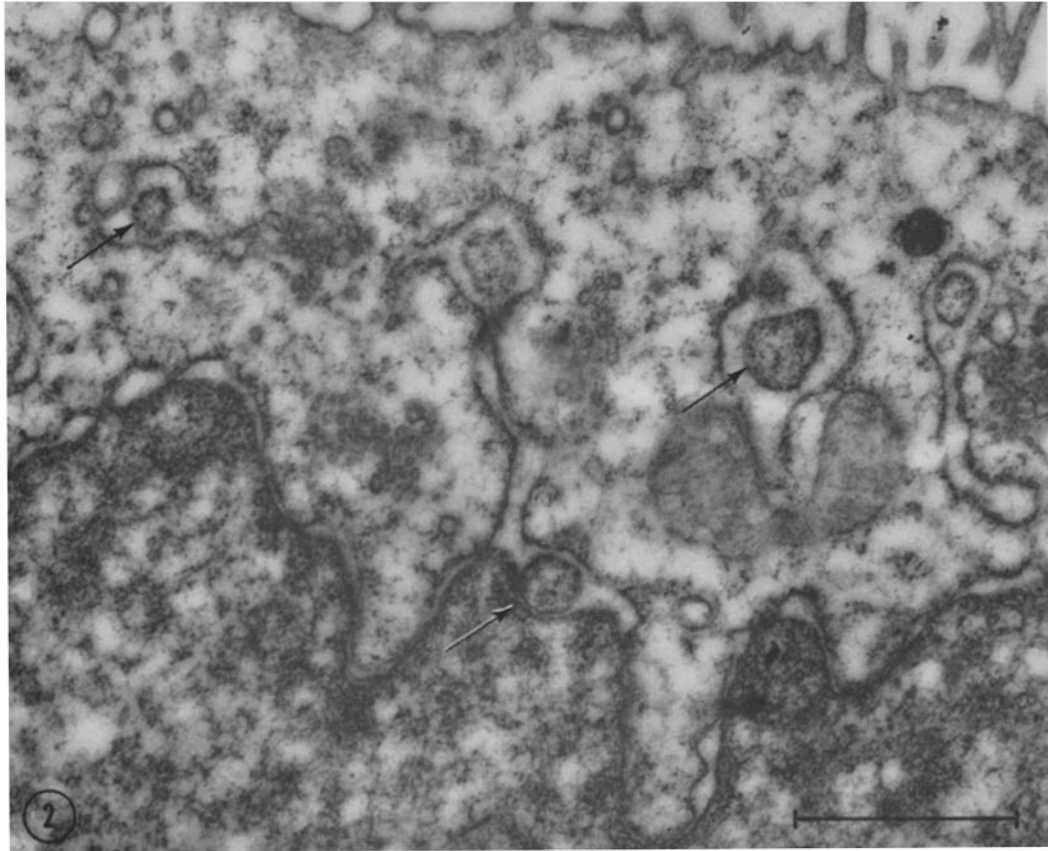
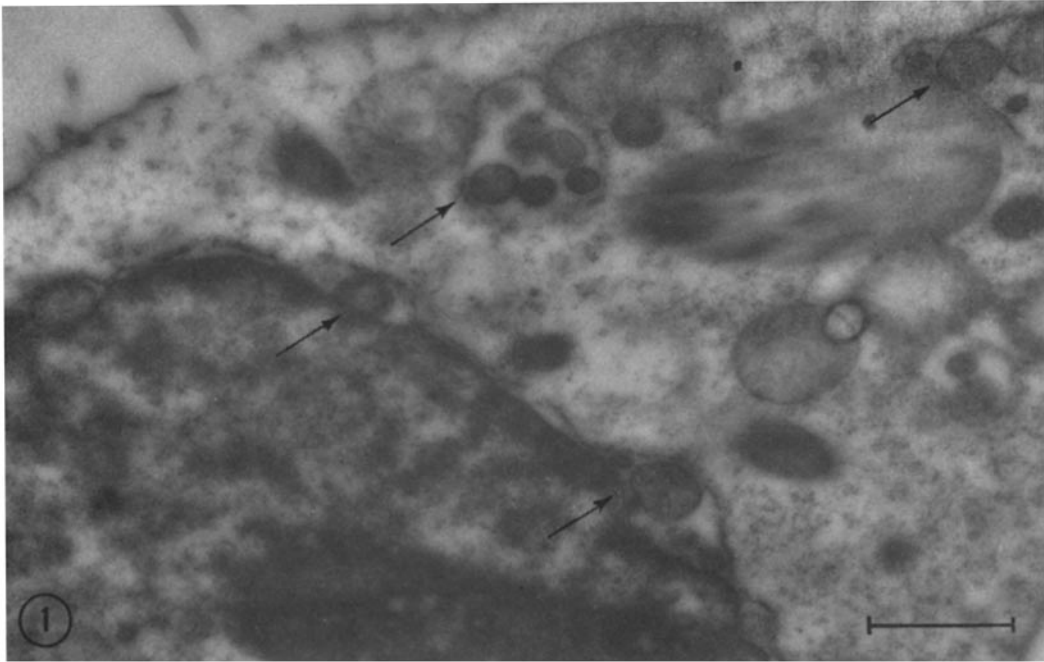
Six days after mating, blastocysts were flushed out of the uterus of New Zealand white rabbits with physiological saline. Immediately after their recovery they were transferred to 1 per cent chilled OsO_4 , buffered with sodium Veronal at pH 7.6 (14). Following 30 to 60 minutes' fixation, the specimens were dehydrated in alcohol and embedded in methacrylate (5:1 butyl-methyl). Some of the sections were stained for 10 to 60 minutes in a saturated aqueous solution of uranyl acetate. Tissues were ob-

served in an RCA EMU-3C electron microscope. This is essentially the same material on which a report has been published describing the presence of intracellular crystalloids within blastocyst cells (8).

OBSERVATIONS

In trophoblast cells of the 6-day rabbit embryo, vesicles of the endoplasmic reticulum frequently contained spherical intracisternal inclusions, up to 0.5μ in diameter (Figs. 1 and 6). These inclusions were membrane-bounded and contained a densely packed granular component quite similar to the free ribosomes abundant in the surrounding cytoplasm. In these cells continuity between cisternal membranes and the outer layer of the nuclear envelope was frequently seen (Fig. 2) and dense inclusions identical to those in the cisternae were present in the perinuclear space between the two layers of the nuclear membranes (Figs. 1 to 5). There can be little doubt that intracisternal and perinuclear spaces were at times confluent, and that the inclusions represent a component common to both.

Of particular interest is the fact that the inclusions in the perinuclear space were frequently in contact with the inner layer of the nuclear membrane (Figs. 4, 5, 7). At such places the inner nuclear membrane appeared to be continuous with the limiting membrane of the inclusions, the region of continuity showing the size and density characteristic of an annulus. Also, like annuli, inclusions were spatially related to interchromosomal areas of the nucleus (Figs. 5 and 7). Occasional points of contact between inclusions and the outer layer of the membrane were also seen (Fig. 3), but these were less common. Similar inclusions, between the two layers of the nuclear envelope, have been seen in several other animal and plant tissues (Figs. 8 to 10).



DISCUSSION

The inclusions described here could arise by the extrusion of nuclear material into the perinuclear space, through those annular openings that penetrate the inner, but not the outer, nuclear membrane. They then could be distributed to the cisternae of the endoplasmic reticulum as they break off the nuclear envelope (Fig. 11). On the other hand, they could arise through the inpocketing of cytoplasmic material into the cisternae. We cannot eliminate the latter possibility in the material at hand, but we favor the view that the inclusions represent the extrusion of nuclear material although the manner in which they acquire their limiting membrane is not clear. Most of the inclusions resemble the interchromosomal areas in the nucleus. They are often more electron opaque than other cytoplasmic structures. Also, when they occur they jam the perinuclear space and are abundant in almost all cisternal cavities in the cell. Adjacent cells may possess similar cisternae, but with the inclusions absent, in which case they are also absent from the nuclear envelope. It thus seems most likely that the inclusions are nuclear, rather than cytoplasmic, in origin.

We have observed this apparent blebbing of nuclear material into the perinuclear space in other cell types (hemocytoblasts of 16-day rabbit embryos, adult hamster epidermis, and cells of the shoot apex in corn), particularly in cells where the perinuclear space and cisternae were enlarged possibly through osmotic imbalance of the medium. These structures also appear in the plasma cell tumors studied by Parsons *et al.* (15), as shown in their Fig. 11. In other cases, cisternae of the endoplasmic reticulum may contain large

spherical inclusions, apparently filled with cytoplasmic materials and derived from inpocketings of the cisternal wall. These have been observed in cells of rat liver after treatment with amino acid analogs (9) and in a hamster melanoma (17). Such structures may be associated with degenerative changes, and appear different from the inclusions described here, both in terms of their size and fine structure.

SUMMARY

Trophoblast cells of 6-day rabbit embryos, as studied with the electron microscope, were observed to contain spherical inclusions up to 0.5μ in diameter inside the cisternae of the endoplasmic reticulum, and also between the membranes of the nuclear envelope. It is suggested that these inclusions arise by extrusion of nuclear material through annular openings in the inner nuclear membrane, and are then distributed to the cisternae as they break off the nuclear envelope.

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FIGURE 1

Portion of trophoblast cell from 6-day rabbit blastocyst. The two upper arrows indicate cisternae of the endoplasmic reticulum, containing numerous spherical inclusions. The two lower arrows indicate similar inclusions between the two membranes of the nuclear envelope. Magnification, 19,000.

FIGURE 2

A cell region similar to that shown in Fig. 1. Arrows indicate inclusions within cisternae, and between membranes of the nuclear envelope. Note the connection between the endoplasmic reticulum and the outer nuclear membrane. Magnification, 29,000.

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FIGURE 3

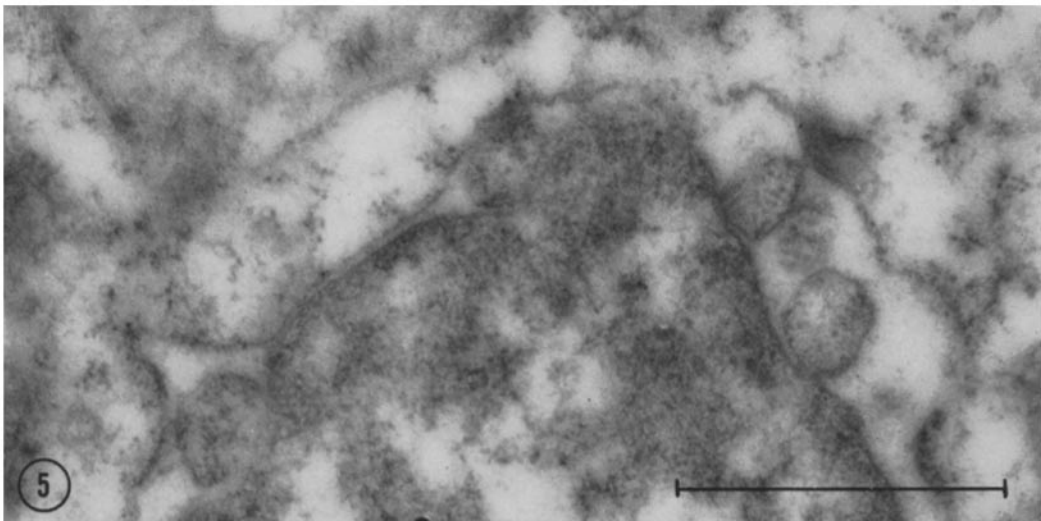
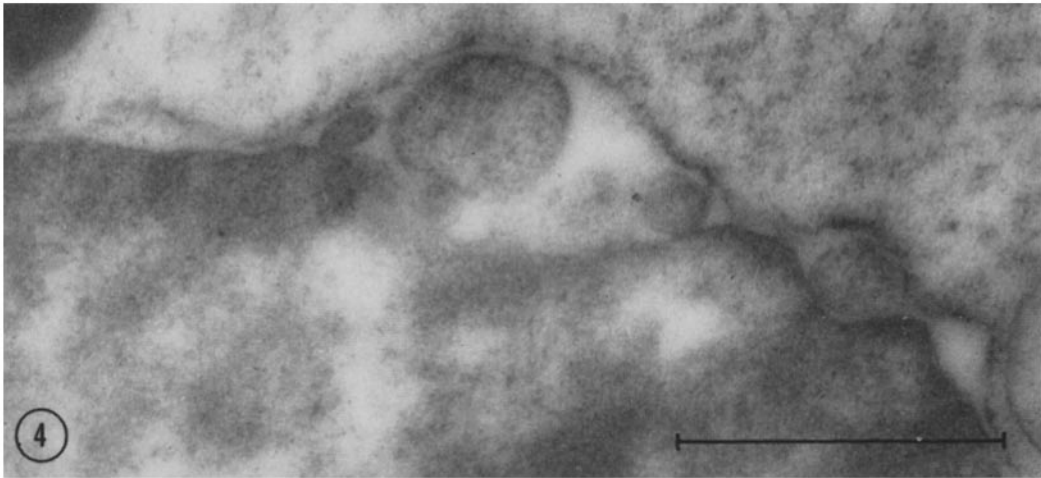
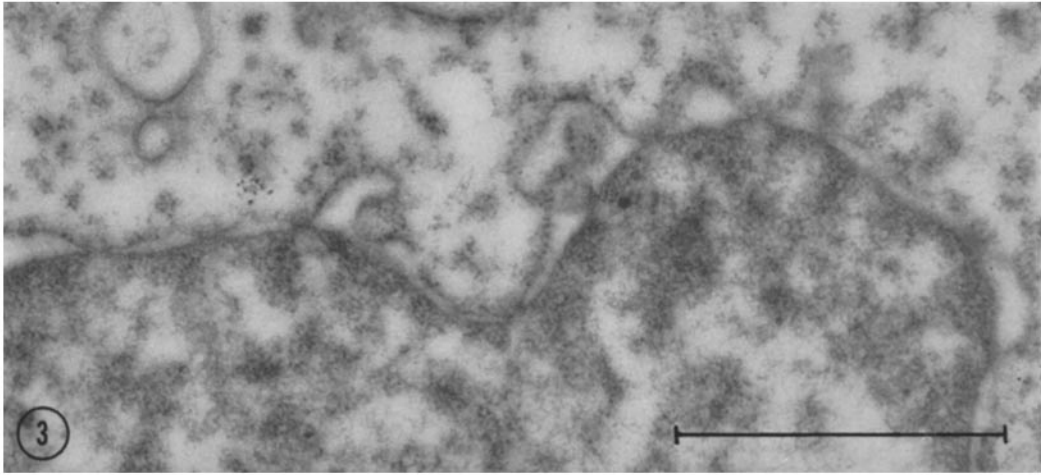
Inclusions between the membranes of the nuclear envelope. In this case the inclusions appear to be in contact with the outer membrane. Magnification, 44,000.

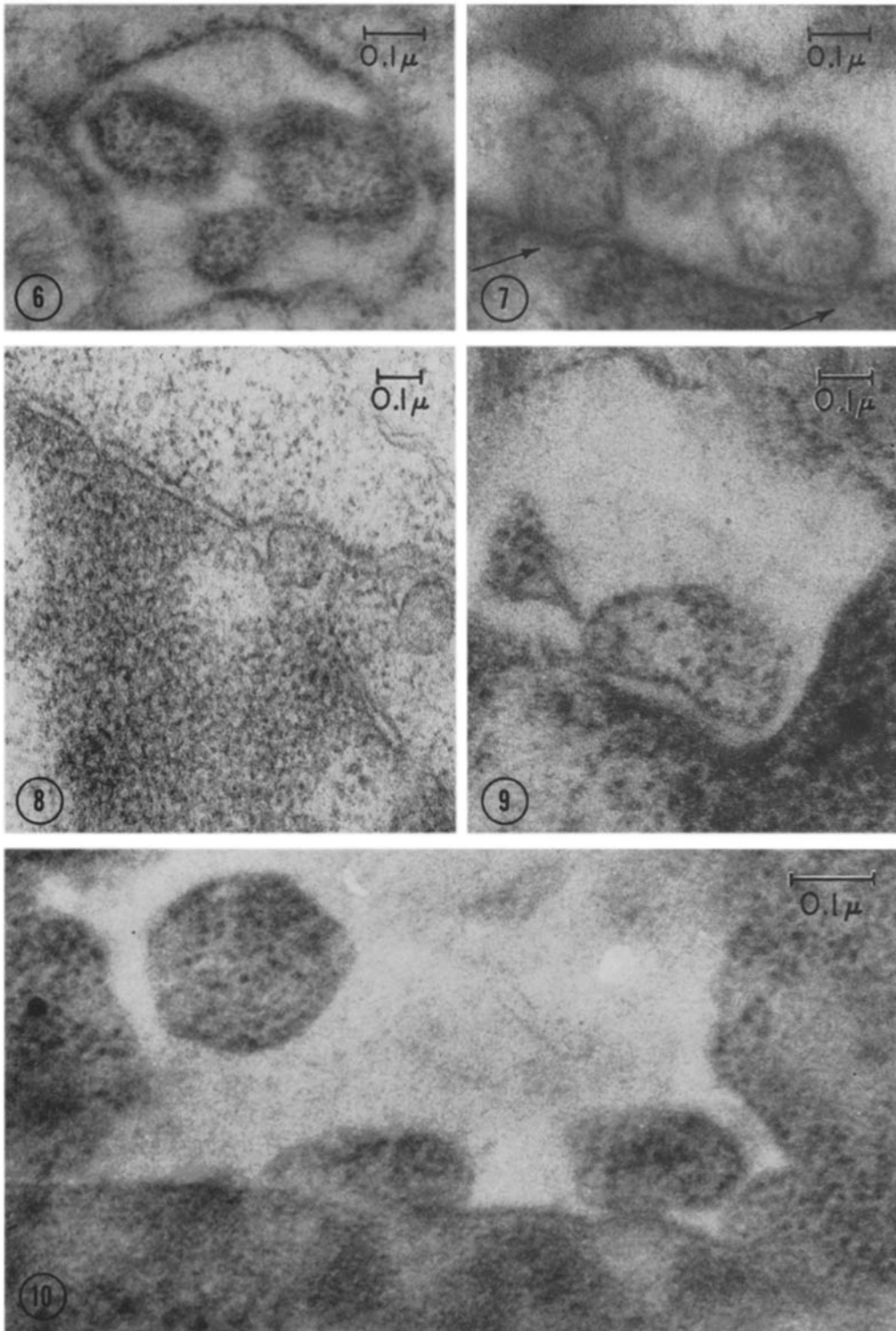
FIGURE 4

Inclusions between the membranes of the nuclear envelope. An enlarged portion of Fig. 1. Magnification, 43,000.

FIGURE 5

Inclusions between the membranes of the nuclear envelope. Magnification, 43,000.





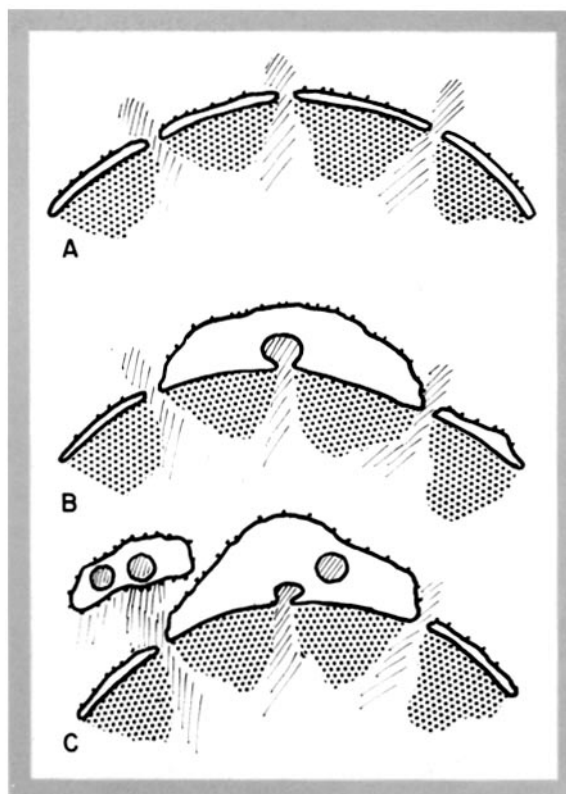


FIGURE 11

Diagram of the nuclear margin showing postulated mechanism of extruded material. *A.* Normal nuclear envelop, showing chromatin area (stippled) and interchromatin areas (cross-hatched). Material passing out of nucleus through annuli is normally dispersed in the cytoplasm. *B.* Expansion of nuclear envelope, possibly by osmotic shock. Opening in outer membrane is apparently closed off. Material passing through annulus is trapped in perinuclear space. *C.* Material leaves its connection with the annulus and lies free in the perinuclear space. The inclusions are carried out into cytoplasm as intracisternal inclusions when portions of endoplasmic reticulum are delaminated from the nuclear envelope.

FIGURE 6

Three inclusions within a small region of the endoplasmic reticulum. Notice the particulate nature of the inclusion contents. Magnification, 86,000.

FIGURE 7

An enlarged portion of Fig. 5, showing continuity between the inner nuclear membrane and the limiting membrane of the inclusions. Also notice the areas of lower density (arrows) beneath the points of continuity, suggesting the inclusions are joined at nuclear membrane annuli. Magnification, 87,000.

FIGURE 8

Portion of nuclear margin from hemocytoblast in liver of 14-day rabbit embryo, showing small inclusion between membranes of the nuclear envelope. Magnification, 58,000; micrograph by J. A. Grasso.

FIGURE 9

Nuclear margin of cell from stratum germinativum of hamster epidermis. The outer membrane of the nuclear envelope is at the top of the picture. Magnification, 83,000.

FIGURE 10

Nuclear margin of cell from the shoot apex of corn (*Zea mays*). The nuclear envelope is widely expanded. Three inclusions are visible between the envelope membranes, two of which are still attached to the nuclear margin below. Magnification, 124,000.